# Lec 4

# Precipitation Reactions

Antibody and **soluble** antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate. Antibodies that aggregate soluble antigens are called **precipitins.** Formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion. Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:

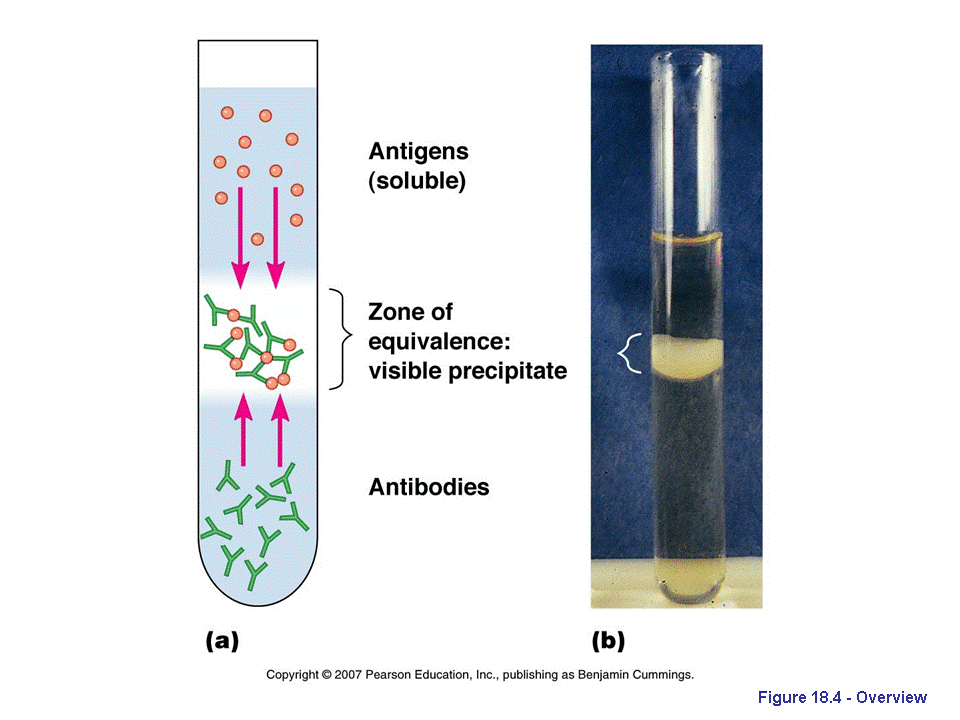
* The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.
* The antigen must be either bivalent or polyvalent; that is, it must have at least two copies of the same epitope, or have different epitopes that react with different antibodies present in polyclonal antisera.

**Precipitation Reactions in Fluids Yield a Precipitin Curve**

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. After the precipitate forms, each tube is centrifuged to pellet the precipitate, the supernatant is poured off, and the amount of precipitate is measured. Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve.



A precipitation curve for a system of one antigen and its antibodies. This plot of the amount of antibody precipitated versus increasing antigen concentrations (at constant total antibody) reveals three zones: a zone of antibody excess, in which precipitation is inhibited and antibody not bound to antigen can be detected in the supernatant; an equivalence zone of maximal precipitation in which antibody and antigen form large insoluble complexes and neither antibody nor antigen can be detected in the supernatant; and a zone of antigen excess in which precipitation is inhibited and antigen not bound to antibody can be detected in the supernatant.



**Precipitation Reactions in Gels Yield Visible Precipitin Lines**

Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in agar, or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form. As in a precipitation reaction in fluid, visible precipitation occurs in the region of equivalence, whereas no visible precipitate forms in regions of antibody or antigen excess. Two types of *immunodiffusion reactions* can be used to determine relative concentrations of antibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation. They are **radial immunodiffusion** (the Mancini method) and **double immunodiffusion** (the Ouchterlony method); both are carried out in a semisolid medium such as agar.

**Radial immunodiffusion**

1. Antibody is added to the gel and poured into a plate, wells are cut into the plate.

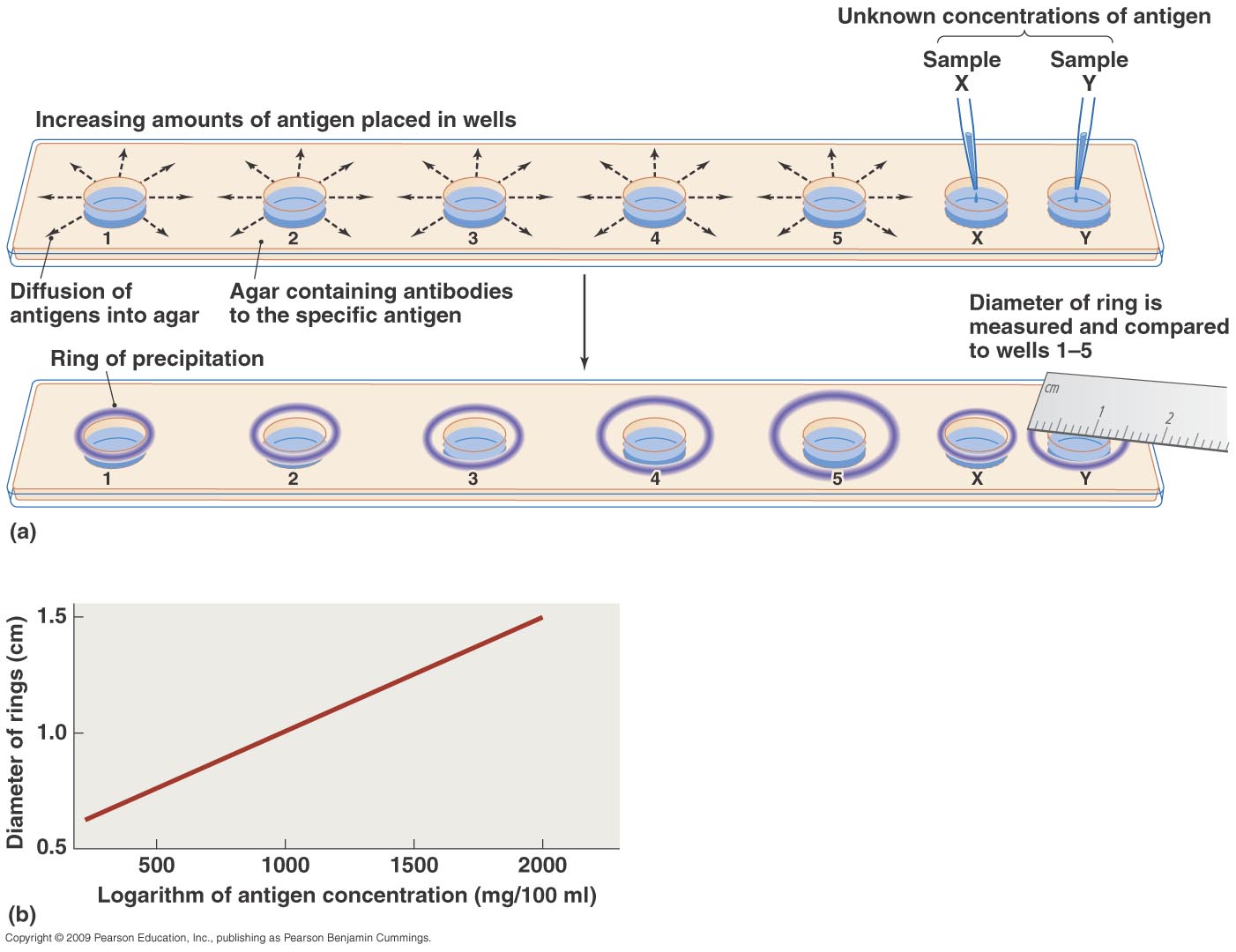
2. Antigen is added to the well and will diffuse out radially from the well.

3. If the antibody present is specific for the antigen added a ring of precipitate will form, the size of the ring is directly proportional to the concentration of the antigen.

4. Standards are run at the same time and a standard curve is created.

By comparing the area of the precipitin ring with a standard curve (obtained by measuring the precipitin areas of known concentrations of the antigen), the concentration of the antigen sample can be determined.





**Ouchterlony method**

Both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equivalence is reached, a visible line of precipitation, a precipitin line, forms (Figure).

1. Holes are cut in the agar, one central hole surrounded by other wells.

2. Antibody is added to the central well, antigens are added to the outer wells, the position of the bands formed between the antigens allows for comparison of the antigens to each other.

3. Suggested antibody and antigen patterns: A straightforward demonstration of identity and non-identity can be shown using the antigen mixtures as in Fig

**Identity**

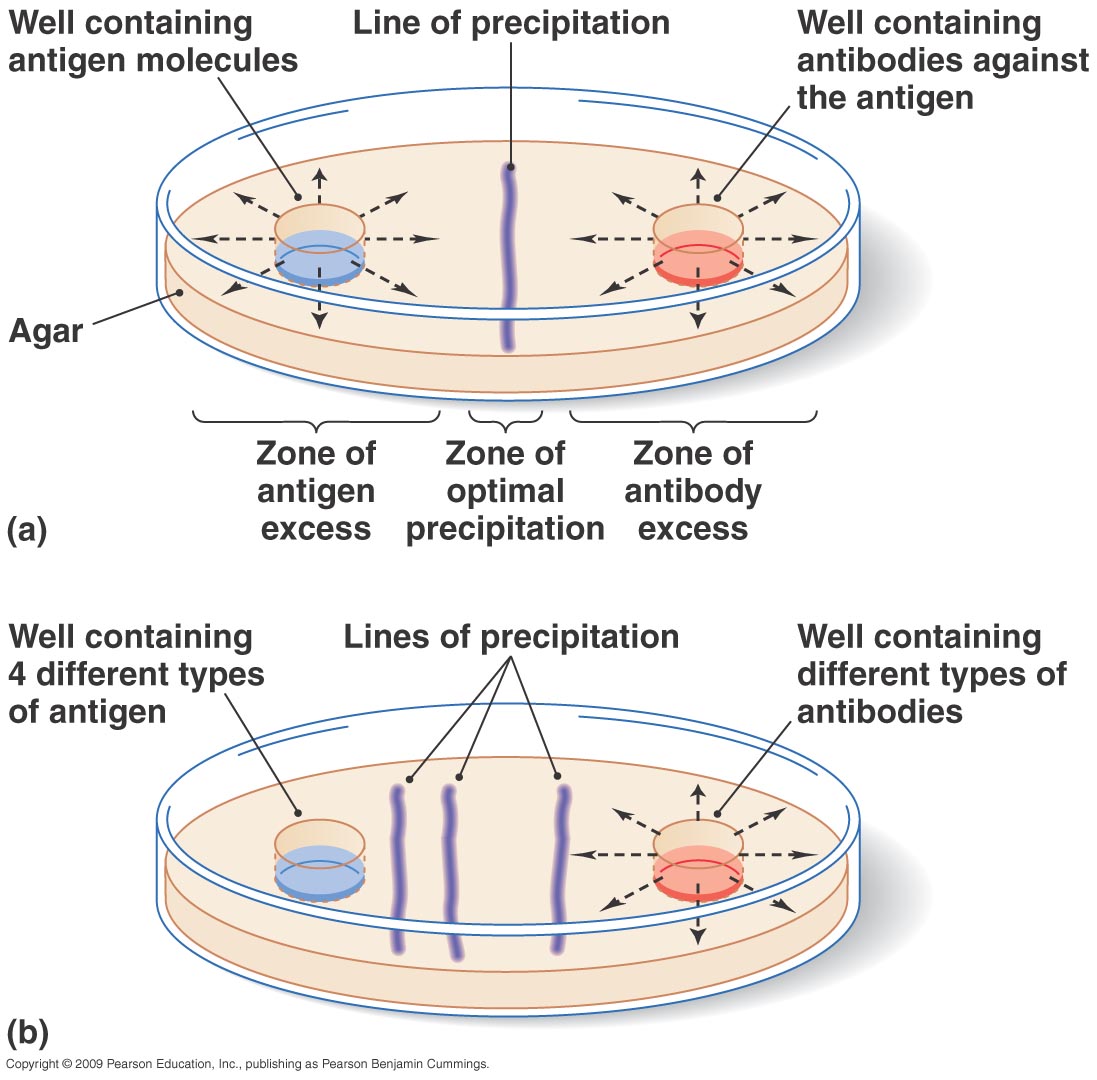
An identity reaction is indicated when the precipitin band forms a single smooth area. This precipitin is formed between the antibody and the two test antigens fuses, indicating that the antibody is precipitating identical antigen specificities in each preparation. This does not mean that the antigens are necessarily identical; they are only identical.

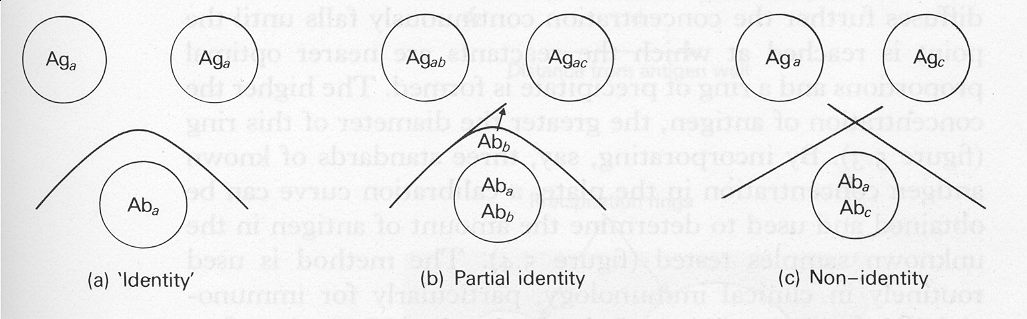
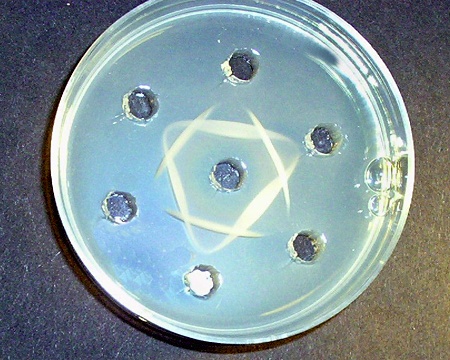
**Nonidentity**

A non-identity pattern is expressed when the precipitation line cross each other. They intersect or cross because the sample contain no antigenic determinants in common.

**Partial Identity**

In a partial identity pattern, the precipitation lines merge with spur formation. This merger indicated that the antigen are non identical but possess common determinants. insofar as the antibody can distinguish the difference.





**Immunoelectrophoresis Combines Electrophoresis and Double Immunodiffusion**

In **immunoelectrophoresis,** the antigen mixture is first electrophoresed to separate its components by charge. Troughs are then cut into the agar gel parallel to the direction of the electric field, and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions (Figure).

Application

* Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A sample of serum is electrophoresed, and the individual serum components are identified with antisera specific for a given protein or immunoglobulin class (Figure).
* This technique is useful in determining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases.
* It can also show whether a patient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin. The immunoelectrophoretic pattern of serum from patients with multiple myeloma, for example, shows a heavy distorted arc caused by the large amount of myeloma protein, which is monoclonal Ig and therefore uniformly charged (Figure).

 Immunoelectrophoresis of an antigen mixture. (a) An antigen preparation (orange) is first electrophoresed, which separates the component antigens on the basis of charge. Antiserum (blue) is then added to troughs on one or both sides of the separated antigens and allowed to diffuse; in time, lines of precipitation (colored arcs) form where specific antibody and antigen interact. (b) Immunoelectrophoretic patterns of human serum from a patient with myeloma. The patient produces a large amount of a monoclonal IgG (λ-light-chain-bearing) antibody.